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THE EFFECT OF SOME TOXIC SOLUTIONS ON MITOSIS

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(WITH SEVEN TEXT FIGURES)

During the two decades just past there has arisen a considerable body of literature from the researches which have been made upon the physiology of the cell under the influence of various external conditions. The effect of certain stimuli on the growth and form of the organism and the modifications in the normal processes of cell and nuclear division induced thereby have been made the basis of numerous generalizations with respect to cellular activities.

Owing to its bearing upon questions of general and practical interest, the action of toxic substances on the growth of plants has been widely investigated. Notwithstanding this fact, our knowledge of the effect of such substances on cellular development in phanerogams is far from extensive, and the nature of toxicity itself yet lacks a satisfactory explanation. What may be called the modern epoch of the toxicity studies began with the researches of KAHLENBERG and TRUE (**10**), in which the action of chemically equivalent solutions of substances experimented with were for the first time compared.

According to the accounts of a number of writers, various departures from the normal course of nuclear and cell division have been caused experimentally by the action of solutions of various chemical constitution. The technic of this type of investigation was first developed by GERASSIMOW in the study of the cells of thallophytes, and later adapted to the study of higher forms.

Numerous investigators have studied the action of ether, chloroform, chloral hydrate, potassium nitrate, phenol, benzol, and copper

sulfate upon cell processes in certain plants, including Spirogyra, Tradescantia, Phaseolus, Lupinus, Vicia, Pisum, Allium, and Larix. The published accounts of these studies show a general agreement as to the production of certain abnormalities in cell and nuclear development. However, as to the occurrence of amitosis, binucleate cells, fusion nuclei with double the normal number of chromosomes and a heterotypic reducing division in the same, opinions are very conflicting.

GERASSIMOW (8, 9), NATHANSON (16, 17), PFEFFER (24), and WASIELEWSKI (30) found amitotic division of nuclei, but WISSELINGH (32, 33), NEMEC (22), ANDREWS (2), and WOYCICKI (34) saw no cases of amitosis.

Binucleate cells were observed by GERASSIMOW (8, 9), NATHANSON (16, 17), PFEFFER (24), NEMEC (18, 20, 21, 22, 23), BLAZEK (3), WASIELEWSKI (30), WOYCICKI (34), and STRASBURGER (29), but they were not found by WISSELINGH (31), ANDREWS (2), and KARPOFF (11).

Fusion nuclei were reported by GERASSIMOW (8, 9), NEMEC (20, 21, 22, 23), who found also double the normal number of chromosomes, and by STRASBURGER (29), but not by the other authors mentioned. The disappearance of the nuclei with the double chromosome number was explained by NEMEC (22) as due to a reduction division, in which he is followed by WOYCICKI (34). This conclusion is severely criticized by STRASBURGER (29) and by LAIBACH (12).

From this brief statement of the conflicting opinions advanced in some of the more important papers dealing with the direct effect of various chemical substances upon nuclear and cell division, it is evident that a much wider range of observation and experiment is required before conclusive generalizations can be drawn or the discordant results of the various investigators be brought into harmony. Moreover, in the higher plants the rate and the amount of growth in the seedling stages have been used almost entirely in the measure of the toxic effect, or when such was not the case some microscopic factor has been employed. Apparently there has been little detailed comparative study of the cell and nuclear activities in the higher plants during the course of treatment with an extended series of dilutions of a toxic agent.

The present investigation was carried out in order to observe the process of nuclear and cell division under certain definite conditions of physiological experiment with a series of toxic substances.

Materials and methods

In this study young seedlings of *Vicia Faba* were used, as they have furnished material for many similar investigations on the physiology of the cell and are well known as suitable material for the study of cellular activities. Uniform series of seedlings selected for the comparative tests were taken from the thoroughly washed sphagnum in which they had germinated, and suspended on glass rods in such a manner that the radicle for about 20^{mm} of its length was immersed in the solution. New nonsol glass beakers of 300^{cc} capacity were used, and a careful distinction was maintained throughout the experiments between those used for the controls and those used for the toxic solutions. This precaution was taken in order to avoid the possibility of the controls being injured by the residuum of copper which, as pointed out by NÄGELI (15), is taken up by the glass and may be given off later to contained solutions in quantity sufficient to affect seriously the radicles of plants grown therein.

The radicles were marked with India ink at a point 15^{mm} from the tip just before they were placed in the solution. When not under direct observation, the seedlings were kept in a dark cabinet in the laboratory at a fairly uniform temperature. The agents used were dilutions made from carefully prepared solutions of copper sulfate, strychnin sulfate, and phenol. The controls in the observations on growth rate were grown in distilled water. Either triple distilled water was used or the water was redistilled from glass just before use. Four seedlings were regularly used in each solution and their average growth was taken as the basis of comparison. Except in a few cases, when one or two tips were dead, four tips from each set of seedlings were prepared for microscopical examination.

In preparing the material for microscopical study, various modifications of Flemming's fluids were used in killing and fixing. Dehydration was followed by imbedding in 53° paraffin. The sections were cut 3–5 μ in thickness and stained on the slide with iron-alum-hematoxylin, safranin-gentian-violet, or the triple stain. A Zeiss

1.5^{mm} apochromatic objective and compensating oculars were used in studying the preparations.

This investigation was begun in 1904, at the suggestion of Dr. RODNEY H. TRUE, to whom I am indebted for constant advice and criticism. I am also under obligations to Dr. G. F. KLUGH, who was of great assistance with the seedling cultures.

Experimental

In all work with the radicles which involved measurement of growth, and in the preparation of material for killing and fixing, great precautions were taken to guard against the introduction of undesirable factors, such as loss of moisture, change in temperature, or shock in handling, which might interfere with the results sought.

THE EFFECT OF COPPER SULFATE SOLUTIONS ON GROWTH

Growth and cell activity in the root tips of *Vicia Faba* upon which copper sulfate solutions of various concentrations had acted continuously were the first subjects of study. Seedlings were placed in a series of solutions with a constant difference of dilution of $n/10{,}000$, in order to determine the concentrations in which the toxic effect would not be so strong as to prevent growth to some degree, after allowing time for recovery from the shock due to the change of medium and for partial acclimatization to the toxic substance. Table I shows the average growth of four seedlings in the various concentrations used, and also the growth made by the corresponding controls in distilled water. From the table it is apparent that, in the lower concentrations, the effect of a difference in dilution of $n/10{,}000$ is masked by the variability of the individual groups of seedlings, and in the higher dilutions this difference was increased many fold before changes in the growth rate were observed which could be reasonably ascribed to the action of the toxic solution. A comparison of the average growth per hour in the copper solution and in distilled water shows that there is a wide range of toxic effect between the concentrations $n/20{,}000$ and $n/500{,}000$, with a probable corresponding difference in cell activity, as shown by the slower rate of elongation in the stronger solutions.

TABLE I

COMPARATIVE GROWTH IN $CuSO_4$ AND DISTILLED WATER DURING THE FIRST DAY.

RADICLES IN COPPER SULFATE SOLUTIONS				CONTROLS IN DISTILLED WATER	
Concentration	Time in solutions in hours	Total growth in mm	Average growth per hour in mm	Growth of corresponding control in mm	Average growth per hour in mm
n/20,000.....	16.5	0.5	....	8.7	0.52
n/30,000.....	19.5	0.5	....	5.0	0.25
n/40,000.....	19.5	1.8	0.09	12.8	0.65
	23.5	1.1	0.04	11.8	0.50
n/50,000.....	19.5	3.0	0.15	12.8	0.65
	20.0	3.7	0.18	7.8	0.39
	21.5	3.8	0.17	11.8	0.54
n/60,000.....	19.5	4.4	0.22	7.8	0.40
	21.5	1.8	0.07	10.2	0.47
n/70,000.....	19.0	8.0	0.42	12.1	0.63
	20.0	6.0	0.30	11.0	0.55
	20.0	5.7	0.28	13.0	0.65
n/80,000.....	18.5	4.5	0.24	11.3	0.61
	19.0	9.2	0.48	12.1	0.63
	20.0	5.9	0.29	13.0	0.65
n/90,000.....	18.5	5.1	0.26	11.3	0.61
	19.0	9.7	0.51	12.1	0.63
	20.0	5.4	0.27	13.0	0.65
n/100,000.....	18.5	7.0	0.37	11.3	0.61
	19.0	11.2	0.58	12.1	0.63
	20.0	8.3	0.41	13.0	0.65
n/110,000.....	18.5	7.0	0.37	11.3	0.61
	21.5	6.5	0.30	14.6	0.67
	24.0	8.5	0.35	14.3	0.59
n/120,000.....	21.5	6.8	0.31	14.6	0.67
	24.0	9.5	0.39	14.3	0.59
n/130,000.....	21.5	8.0	0.37	14.6	0.67
	24.0	12.3	0.51	14.3	0.59
n/140,000.....	21.0	5.0	0.23	8.3	0.39
	24.0	11.8	0.49	16.0	0.66
n/150,000.....	21.0	6.5	0.30	8.3	0.39
	22.0	13.5	0.61	16.5	0.75
n/160,000.....	24.0	12.8	0.53	16.0	0.66
n/250,000.....	19.0	11.5	0.60	14.2	0.74
	19.0	8.8	0.46	10.3	0.54

TABLE I—*Continued*

Radicles in copper sulfate solutions				Controls in distilled water	
Concentration	Time in solutions in hours	Total growth in mm	Average growth per hour in mm	Growth of corresponding control in mm	Average growth per hour in mm
n/275,000....	19.0	11.8	0.61	14.2	0.74
	19.0	11.0	0.57	10.3	0.54
	18.0	7.8	0.42	15.0	0.83
n/300,000....	19.0	11.2	0.58	14.2	0.74
	19.0	10.3	0.54	11.5	0.60
	19.0	14.0	0.71	10.3	0.54
	18.0	6.0	0.33	15.0	0.83
n/400,000....	19.0	10.0	0.52	11.5	0.60
	18.0	12.7	0.70	15.0	0.83
	24.0	14.8	0.61	15.8	0.65
n/500,000....	18.0	16.0	0.88	15.0	0.83
	24.0	13.7	0.57	15.8	0.65

Observations were next made on the growth rate to ascertain whether the retardation occasioned a gradually increasing lag in rate of elongation, or an abrupt termination of growth due perhaps to the sudden failure of vitality in the cell. Table II shows the average growth of several groups of four radicles, each selected from the various concentrations, and of the corresponding controls in distilled water. The several growth periods recorded for each concentration were consecutive.

With one exception, growth was in every case less in the copper sulfate solution than that made by the control in distilled water. In the higher concentrations growth was practically inhibited at the end of a twenty-four hour period. Passing down the series the growth is seen to be gradually diminished after the first twenty-four hours. A similar reduction in growth rate is apparent in the controls, though in a degree much less marked.

The observations summarized in tables I and II indicated that the series of concentrations selected would afford material showing strong toxic action resulting ultimately in death (*n*/20,000 to *n*/50,000), as well as the more prolonged and gradual though no less fatal effect of higher dilutions.

TABLE II

AVERAGE GROWTH IN COPPER SULFATE SOLUTIONS AND DISTILLED WATER*

COPPER SULFATE SOLUTION			DISTILLED WATER
Concentration	Time in solution in hours	Growth in mm	Growth of corresponding control in mm
n/50,000	21.5	2.3	10.2
	24.5	0.0	8.0
	49.0	0.0	11.6
n/90,000	20.0	4.5	11.0
	24.0	1.2	12.3
n/110,000	18.5	7.0	11.3
	25.0	2.0	11.7
	22.5	0.5	9.7
n/150,000	21.0	6.5	8.3
	22.0	4.0	10.2
	24.0	0.7	10.2
n/250,000	19.0	11.5	14.2
	24.0	9.2	14.0
	24.0	3.7	11.2
n/300,000	19.0	11.2	14.2
	24.0	5.7	14.0
	24.0	0.5	11.2
n/400,000	18.0	12.7	15.0
	24.0	10.5	13.0
	24.0	4.2	10.0
n/500,000	18.0	16.0	15.0
	24.0	15.2	13.0
	24.0	7.2	10.0
n/500,000	24.0	13.7	17.2
	24.0	7.5	14.7
	24.0	1.5	2.7
	24.0	0.5	3.0

*The intervals under each concentration represent consecutive periods of exposure for a single group of seedlings. The sum of these intervals will give the total time in the solution.

THE EFFECT OF DILUTE SOLUTIONS OF COPPER SULFATE ON MITOSIS

The material discussed in this section was selected from cultures in various concentrations of copper sulfate, and the root tips chosen for study, together with the corresponding control in distilled water, were fixed in the manner noted above at intervals of approximately twenty-four hours. Some preparations were made at other intervals in order to increase the range of observations.

In root tips fixed after an exposure of one hour to $n/20{,}000$, mitosis was arrested; after 16 hours the root tips were dead. After 20 hours in $n/30{,}000$ there were no mitoses, and the cells of the dermatogen, outer periblem, and meristem were dead and disintegrating. The nuclei of the cells not disorganized were of normal size and occasionally contained two nucleoli.

After exposure for 40 hours to $n/40{,}000$ there were only rare mitoses. The outer cell layers were plasmolyzed, the mid-plerome cells were vacuolate, and the persistent nuclei shriveled. The nuclei of the larger number of the other cells of the plerome were resting and very frequently contained two nucleoli. The apex of the tip was dead, but some development in the plerome region was evidenced by the thickened walls of the cells destined to form the fibrovascular bundle. These thickened cells extended down to within 2^{mm} of the end of the radicle. At 3^{mm} from the apex of the tip there was an area of hypertrophied periblem cells (*fig. 1*) which had developed to several times the normal size, producing distortion of the radicle and giving it a swollen edematous appearance on one side. Since the nuclei in these cells were disorganized, a corresponding increase in size could not be determined.

In tips exposed for 6 hours to $n/50{,}000$ there were a few division figures. An exposure of 20 hours to this concentration killed all the cells in the meristem region. Many of the middle periblem cells were greatly enlarged, and in the others practically all nuclei were in the resting stage and were rich in chromatin. Cells with two nucleoli were very common in the plerome region, where also a few cells were observed containing three nucleoli. In general appearance the plerome cells resembled those shown in *fig. 2b*. After 44 hours practically all the cells were dead and disintegrating.

An exposure of 20 hours to $n/70{,}000$ killed the outer layers of cells, but in the inner periblem normal chromatic figures were present, and as in the preceding cases the achromatic figures were obscure. At the end of 46 hours large vacuoles appeared in the cytoplasm of many cells, and frequently so crowded upon the nucleus that it was driven to one side of the cell. Nearly all of these cells were enlarged in size and irregular in outline. In the inner periblem practically all stages of the chromatic figure occurred, although very few cells

contained other than resting nuclei. After 69 hours' exposure to this concentration the older periblem cells were still more enlarged and the outer ones were disintegrating. A few of the inner periblem cells showed division stages, none of which were later than early anaphase.

The cells of the root tips treated with $n/190{,}000$ for 44 hours differed little from those in $n/70{,}000$ for the same length of time.

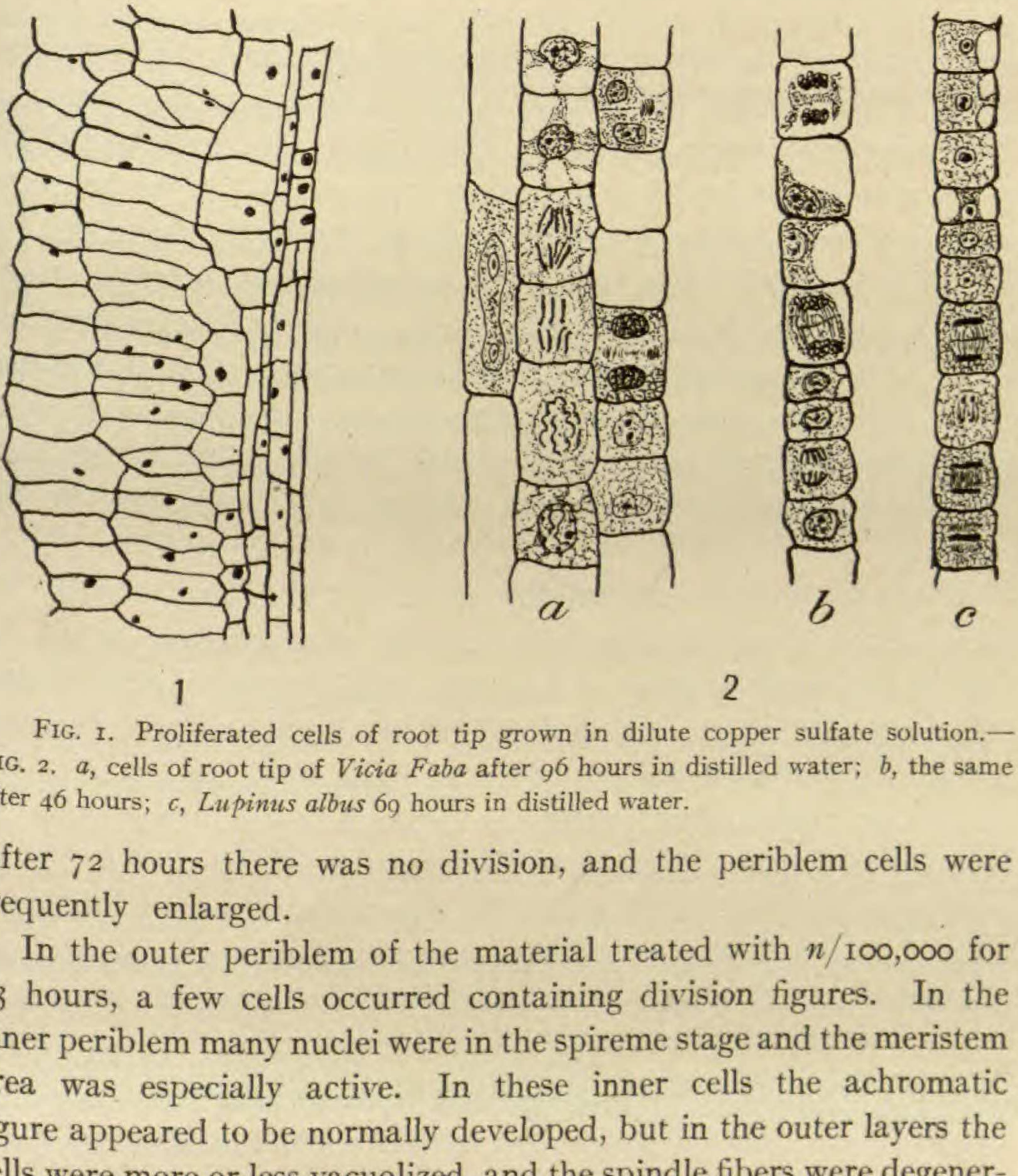


FIG. 1. Proliferated cells of root tip grown in dilute copper sulfate solution.—FIG. 2. *a*, cells of root tip of *Vicia Faba* after 96 hours in distilled water; *b*, the same after 46 hours; *c*, *Lupinus albus* 69 hours in distilled water.

After 72 hours there was no division, and the periblem cells were frequently enlarged.

In the outer periblem of the material treated with $n/100{,}000$ for 43 hours, a few cells occurred containing division figures. In the inner periblem many nuclei were in the spireme stage and the meristem area was especially active. In these inner cells the achromatic figure appeared to be normally developed, but in the outer layers the cells were more or less vacuolized, and the spindle fibers were degenerating. In 93 hours this condition had spread to all the cells.

Approximately similar conditions occurred in the radicles treated in the concentrations $n/140{,}000$ and $n/150{,}000$. In 93 hours prac-

tically all cells were dead, the outer layers being also disorganized. However, in the case of the material in $n/150{,}000$ for 93 hours, in the periblem cells about 3^{mm} from the apex of the root tip a few mitotic figures occurred. In the corresponding controls in distilled water, chromatic figures were frequent even in the outer periblem cells, but many abnormalities occurred in the achromatic figures and cytoplasm (*fig. 2b*). Nuclear division occurred here and there in the inner periblem of tips which had been 93 hours in a $n/160{,}000$ solution, but the outer periblem was dead and disorganized. The division figures did not differ from those in the controls, and the nuclei in the resting stage were normal in appearance. In tips exposed for 68 hours to the action of $n/300{,}000$, $n/400{,}000$, and $n/500{,}000$ solutions, division was frequent in all parts of the active tissue and did not differ essentially from that in the corresponding controls. However, the outer cell layers of the tips in the copper solutions were less active, and the total number of mitoses was smaller than in the controls. The cytoplasm of many cells contained vacuoles of variable size, some being so large as to crowd heavily upon the nucleus. The same phenomenon occurred, though less frequently, in the controls.

DEMOOR (7), who studied the effect of chloroform on the cell protoplasm, observed therein a marked vacuolization which he regarded as the direct result of the action of this reagent. Also NEMEC (18) found that chloroform and potassium nitrate produced vacuoles in both chromosomes and cytoplasm of *Vicia Faba*, and BLAZEK (3) states that benzol vapor caused the vacuoles of the cytoplasm so to increase in size that they caused deformation of the nuclei. However, since vacuoles, similar to those observed in the course of these experiments with copper sulfate, occurred also in the controls in distilled water, it would appear to follow that this phenomenon is not necessarily due to a narcotic or poisonous action, but may result from an alteration in the concentration of the cell fluids.

Before the examination of the toxicated material had proceeded very far, it became evident that the conditions of mitosis in the controls grown in distilled water were far from normal. The entire series of controls was thereupon reexamined, and a progressive degeneration demonstrated therein closely resembling that in the cells treated with

the copper solutions. After 7 hours in distilled water there was little division in the periblem, the figure in a few cells being in early prophase. Division was active in the plerome. After 22 hours division had ceased in the outer layers, but still occurred normally in the inner tissues. In 46 hours the radicles were curved, numerous cells were dead, large vacuoles occurred in the cytoplasm, and in many cells the achromatic figure was degenerating (*fig. 2b*). The general condition after 96 hours is illustrated in *fig. 2a*. Particularly striking are the large nuclei, the cytoplasmic vacuoles, and the interrupted cell plate seen in one of the upper cells of the figure. *Fig. 2c* represents a group of cells from a radicle of *Lupinus albus* after 46 hours' exposure to distilled water, and is inserted here for the purpose of comparison. In these cells also vacuoles occurred in the cytoplasm, and there was some degeneration of cytoplasmic structure.

It appears that the distilled water exerted practically the same effect on mitosis as was produced by the dilute copper sulfate solutions, but only after a more prolonged exposure.

THE EFFECT OF MORE CONCENTRATED SOLUTIONS OF COPPER SULFATE

In planning the experiments with stronger solutions of copper sulfate, some paragraphs from NEMEC's "Ueber ungeschlechtliche Kernverschmelzungen" (**21**) were held in mind. In this paper NEMEC describes the production of binucleate cells and other abnormalities by placing the radicles of *Vicia Faba* for thirty minutes in 1 per cent solution of copper sulfate and then transferring them to moist sawdust for seven hours. NEMEC's experiment seemed to indicate that *Vicia Faba* was remarkably resistant to the action of copper solutions, indeed to a far greater degree than in *Lupinus albus*, in which, as was learned through access to some unpublished notes of Dr. R. H. TRUE, some growth can occur after an exposure of eight minutes' duration to a $n/16$ solution of copper sulfate. A test quickly showed that thirty minutes' exposure to a 1 per cent copper sulfate solution (approximately $n/12$) was fatal to the material being used in these experiments. A series of preliminary experiments was carried out, therefore, to establish approximately the time limit

which would just permit growth in $n/4$ and $n/12$ solutions as boundary concentrations, with results indicating that a slight amount of growth would follow exposure to a $n/4$ solution for three minutes, and that seven minutes' exposure to the $n/12$ solution, while permitting some growth, was not far from the point of killing.

For the experimental work the solution $n/12$ was chiefly relied upon as being best suited to give a sharp toxic effect, without endangering the loss of the material through death. The radicles were exposed to this solution for periods of three and seven minutes, respectively, were then rinsed quickly in distilled water, and at once transferred to the medium in which they were kept until the time for killing and fixing. Another dilution and longer interval of exposure were also employed to furnish a broader basis for observation.

In order to have at hand for constant comparison material grown under parallel conditions with the toxicated radicles, except for the treatment with the copper sulfate solutions, a uniform lot of seedlings was taken from the germinating chamber, selected to approximately the same size, and divided into six groups which were then prepared as follows: (*a*) one group in moist sphagnum; (*b*) one in distilled water; (*c*) one in $n/12$ copper sulfate three minutes, then sphagnum; (*d*) one in $n/12$ copper sulfate three minutes, then distilled water; (*e*) one in $n/12$ copper sulfate seven minutes, then sphagnum; (*f*) one in $n/320$ copper sulfate ten minutes, then distilled water.

Four root tips were fixed from each series at intervals of 3, 7, 22, and 30 hours. Sections of the tips from the first two series, designated as the controls in the following pages, were compared at every stage with the sections studied in the remaining series.

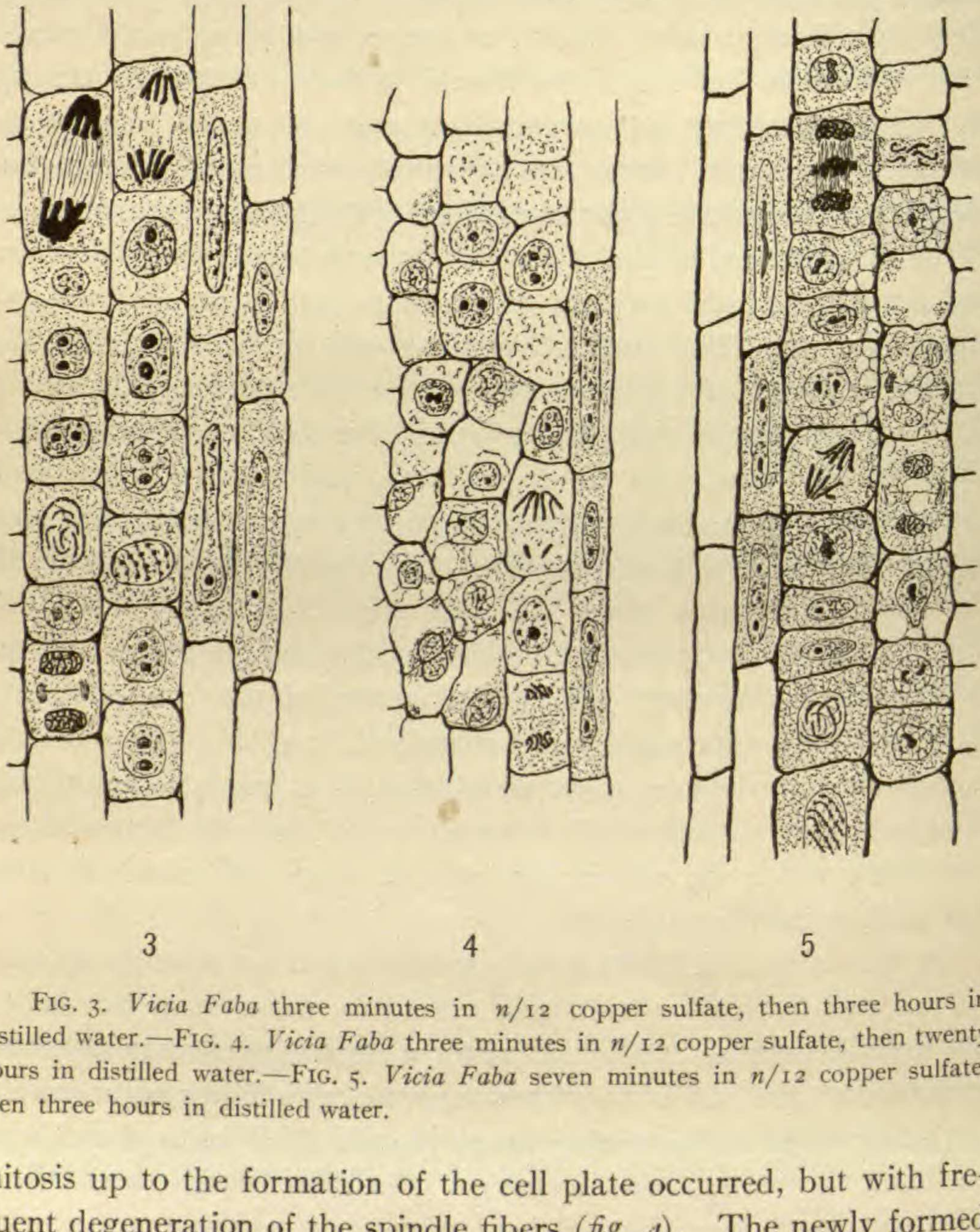
The cell growth and nuclear division observed in the controls placed in sphagnum were considered as normal, since good preparations were secured showing abundant mitoses, all of which conformed to the type generally reported as occurring in vegetative tissues of *Vicia Faba* (*fig. 6*). Departures from the normal cell division were observed in the controls grown in distilled water similar to those observed in the controls paralleling the cultures in dilute copper sulfate solutions (*figs. 2a, 2b*). Since the radicles in the copper sulfate solutions were manipulated under conditions identical with those obtaining in the controls, except for the exposure to the toxic solution,

marked discrepancies in nuclear behavior, it was believed, could safely be regarded as a result of toxic action.

Root tips which were exposed to *n*/12 copper sulfate solution for three minutes and then transferred to distilled water for three hours showed the effect of strong toxic action in the peripheral cell layers. Here the cells were dead, but in the inner periblem occasional nuclei were in division, but frequently the cell plate had failed to form (*fig. 3*). In the majority of the uninjured cells the nuclei were in the typical resting stage. In no case did nuclei which were in the spireme stage show the hyaline polar caps, frequently figured as characteristic of the development of the normal achromatic figure in *Vicia Faba*, and occurring in the controls grown in sphagnum in these experiments (*fig. 6*). The nucleoli were usually large and frequently occupied a clear area surrounded by the linin network, as seen in *fig. 3*. Many nuclei contained two large nucleoli, each lying within a distinct clear area. In the degenerating nuclei of the injured cells the persistent nucleolus was generally of very large size, and the form of the nucleus was usually outlined only by the linin network, all chromatic substances other than the nucleolus having lost their usual staining properties. In some cells the cytoplasm showed modifications apparently due to the action of the copper sulfate, although these were usually not sharply defined, but in some cases numerous large vacuoles occurred distributed through the cytoplasm, in others huge vacuoles had formed at the sides of the nucleolus, presenting much the appearance of the older cells in which large sap cavities had formed.

In root tips which had been exposed for three minutes to *n*/12 copper sulfate solution and then placed in water for seven hours, the cells presented much the same general appearance as those examined at the end of the three-hour period. The greater part of the nuclei were in the resting stage, though a few cells of the inner periblem showed spiremes forming. Occasional nuclei were farther advanced in division, but no stage later than middle anaphase was seen. In the distribution of the chromosomes, and in their manner of passing from the nuclear plate, no deviation from the typical process occurring in the controls in sphagnum was observed. After 22 hours a decided change in the appearance of the cells of the root

tip was apparent. The dermatogen had exfoliated and the periblem cells, having lost their normal rectangular form, were irregularly rounded. All early stages of mitosis were present, and the development of the chromosomes had proceeded normally. All stages of



3 4 5

FIG. 3. *Vicia Faba* three minutes in *n*/12 copper sulfate, then three hours in distilled water.—FIG. 4. *Vicia Faba* three minutes in *n*/12 copper sulfate, then twenty hours in distilled water.—FIG. 5. *Vicia Faba* seven minutes in *n*/12 copper sulfate, then three hours in distilled water.

mitosis up to the formation of the cell plate occurred, but with frequent degeneration of the spindle fibers (*fig. 4*). The newly formed division walls separating certain cells were very thin and indistinct, and the appearance presented on first examination suggested the binucleate cells described by NEMEC (**22**) and others. These division walls were usually asymmetric with the other cell walls. In

the root tips examined after 30 hours, division still occurred in the periblem cells. The chromatic figure was regularly found, but only in the inmost layers of cells was the spindle unaffected. The nuclear plate had formed in the central spindle region of most cells, but usually failed to reach the walls.

In the root tips placed in distilled water for three hours after seven minutes' exposure to $n/12$ copper sulfate, very few mitoses occurred in the periblem cells, but in these the chromatic figure presented a normal appearance. The cytoplasm of all the outer periblem cells exhibited large vacuoles, some of which had enlarged sufficiently to drive the nucleus to one side of the cell, and in the inner periblem frequent cells developed unusual vacuoles. In some cells in late anaphase vacuoles occurred between the cell plate and the daughter nuclei (*fig.* 5), a condition which occurred also in the distilled water controls, but division did not proceed farther in the copper solution. At one side of some cells the plate, failing to reach the lateral wall, ended in a fibrous plasma mass about half-way between the axis of the spindle and the wall of the cell. In the cells at the apex of the root tip occasional nuclei were in early prophase, anaphase was frequent, and a few nuclei were at telophase. In the latter stage the cell plate had formed in the normal manner, but in practically all the nuclei at anaphase no trace of cell plate appeared. In some cells the spindle fibers were only faintly visible, in others a perceptible thickening of the fibers had occurred in the equator of the spindle, but no figure showed the line of granules characteristic of cell plate formation. In the resting nuclei, as usual, there occurred one or two large nucleoli. These were rarely circular as viewed in optical section, but were amoeboid in form.

In radicles placed for ten minutes in $n/320$ copper sulfate, then transferred to distilled water for three hours, the dermatogen cells were dead and many of the outer periblem cells lacked nuclei. The nuclei present were in the resting stage. Numerous mitoses occurred in the cells of the plerome and inner periblem, but the larger number showed a tendency toward degeneration in the spindle fibers. After seven hours the general appearance of the cells was much the same, but there were very few mitoses. After 22 and 30 hours, respectively, no division figures occurred in the outer cell layers, but a few cells

in the periblem and inner plerome still showed normal chromatic figures; some nuclei were in the spireme stage, although the majority were resting; cellular activity, as expressed in division, had practically ceased.

A comparative estimate of the proportion of cells in course of division in each of the several experiments did not show that any concentration used had stimulated division; on the contrary, the retarding influences, particularly in the more concentrated solutions, were very pronounced. The first apparent effect of the toxic solution was arrest of nuclear division through inhibition of the activities of the achromatic figure. In the early division stages this was soon followed by degeneration of the spindle fibers. In the later stages of division the failure of cell plate formation was characteristic. These phenomena were accompanied or followed by an increase in the number and size of the vacuoles in the cytoplasm. The death of the cell evidently occurred shortly after this condition was reached.

It seems probable that the toxic solution penetrates somewhat slowly to the inner cell layers, since under its influence the outer layers of cells are killed, while in the inner regions not yet visibly affected, normal development continues.

There was no satisfactory evidence of the occurrence of amitosis. Double nucleoli occurred as frequently in the cells of the controls as in those treated with the copper solutions, a result which, so far as these experiments have extended, directly controverts the statement of WASIELEWSKI (**30**) that "das erste Kennzeichen, dass ein Kern sich zur amitotischen Theilung anschickt, besteht in einer Verdoppelung des Nucleolus."

The copper solutions did not cause abnormalities in the development of the chromatic figure. There was no doubling of the normal number of chromosomes. Occasionally two daughter chromosomes remained attached by their ends for some time after the others had left the nuclear plate, apparently forming an attachment between the daughter chromosome groups. However, this irregularity was also observed in the controls. NEMEC (**21**) states that treatment with 1 per cent copper sulfate solution produced binucleate cells in root tips of *Vicia Faba*. After 17 hours' sojourn under normal conditions binucleate cells no longer appeared, and he concluded, therefore, that

the nuclei in the binucleate cells had fused. The experiments here described furnish no support to this theory of nuclear fusion, since no cells were observed that contained more than one nucleus. Occasionally, through failure of the nuclear plate, cells appeared to contain two nuclei, but these daughter nuclei were never fully reconstituted, and the cells were degenerating.

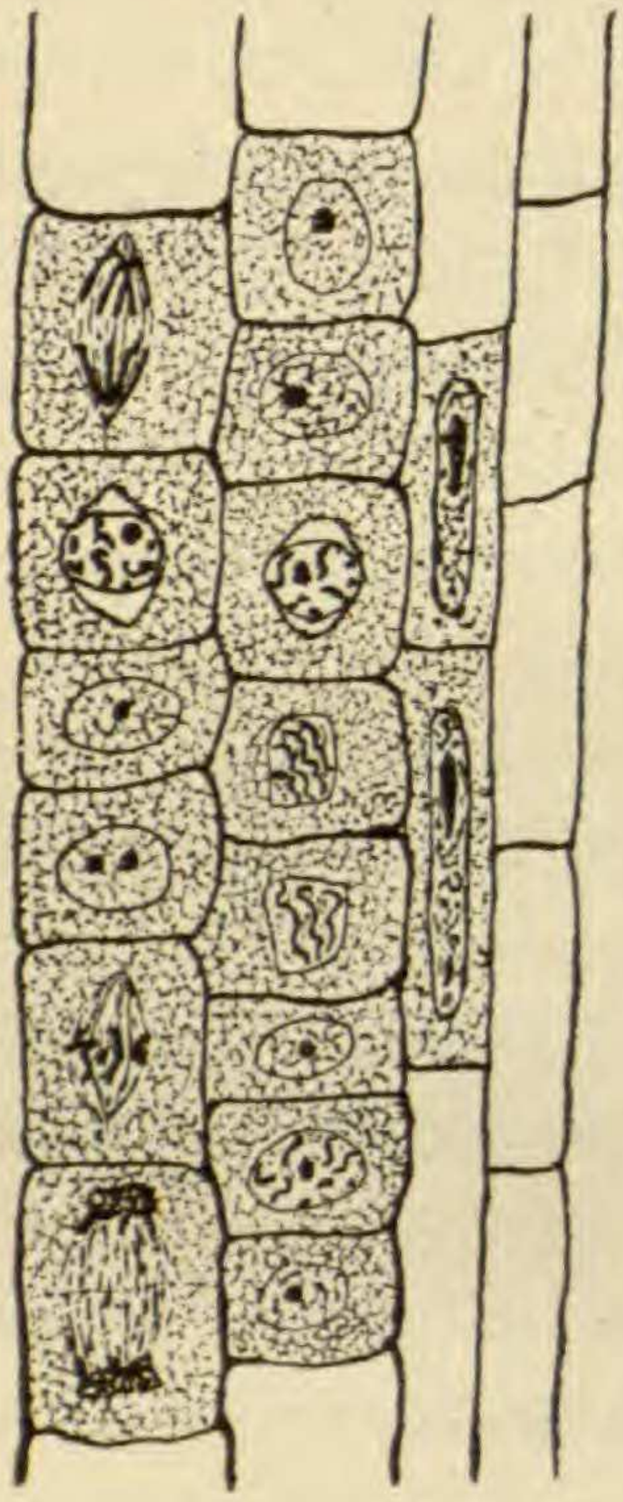
The stronger copper solutions inhibited mitosis, disorganized the spindle fibers or interrupted their formation, arrested the development of the cell plate, and produced large vacuoles in the cytoplasm. The same effects were produced in the controls in distilled water, though to a less marked degree, and after a longer period of exposure. There were no abnormalities in the controls grown in sphagnum.

THE ACTION OF PHENOL

In studying the action of phenol a normal solution was prepared, and various dilutions were made therefrom in the course of the experiment. Controls were grown in moist sphagnum and in distilled water. The continuous action of phenol was observed in $n/94$ and $n/188$ solutions, respectively. Solutions of 10/94, 5/94, and 1/188 normal were allowed to act on radicles for 20 minutes, after which they were placed in distilled water and material killed and fixed therefrom at intervals of 4, 21, and 45 hours. Material from the controls received parallel preparation. In the microscopical examination of the toxicated root tips no unusual structure or condition was considered as due to the action of the phenol until careful search had shown that its equivalent did not exist in the controls.

The continuous action of a $n/94$ solution of phenol for four hours seriously injured both the cytoplasm, which showed numerous small vacuoles, and the achromatic portion of the nucleus. Numerous spireme nuclei were observed, many of which were much enlarged and irregularly distended apparently by a great increase in the amount of nuclear sap within them. Occasionally these enlarged spireme nuclei were laterally indented by the formation of a dense plasma mass at one side (*fig. 7a*). These nuclei very much resembled those described by NEMEC (**22**) as formed under the influence of chloral, of which he says: "In einiger Zellen giebt es mehr oder weniger tief eingeschnürte Kerne." At this point the resemblance ceases, and no support can

be given to NEMEC's further statement that "diese Zellen können Scheidewandanlage besitzen." Since no other unusual forms were observed in either cell or nucleus, these are regarded as nuclei in the course of disorganization. In the older as well as in the younger portions of the root tip nuclear figures occurred which usually showed



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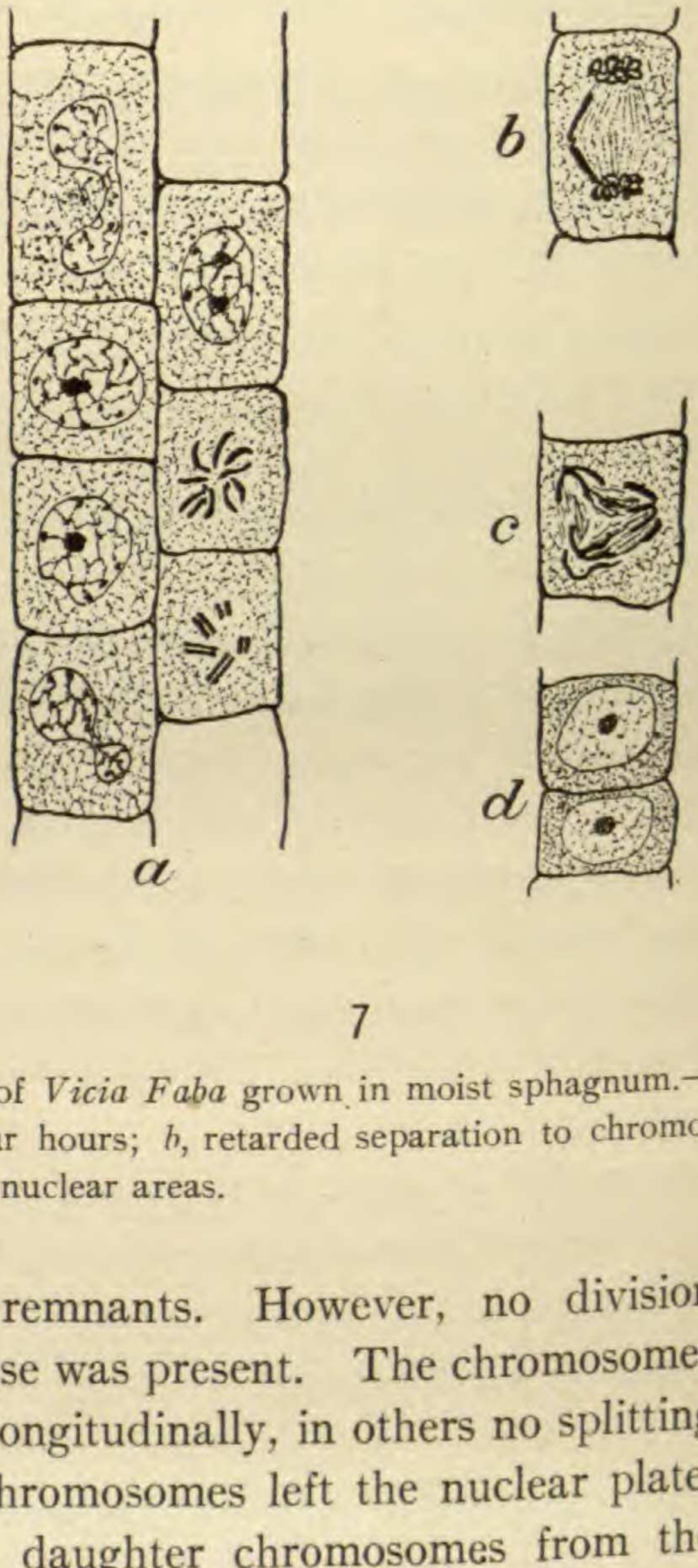

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FIG. 6. Normal cells from a root tip of *Vicia Faba* grown in moist sphagnum.—FIG. 7. *a*, *Vicia Faba* in *n*/94 phenol four hours; *b*, retarded separation to chromosomes; *c*, multipolar spindle; *d*, enlarged nuclear areas.

the achromatic spindle or its remnants. However, no division stage later than very early anaphase was present. The chromosomes in part of these figures had split longitudinally, in others no splitting occurred. In no case had the chromosomes left the nuclear plate. Evidently the movement of the daughter chromosomes from the nuclear plate to the poles of the spindle had here been inhibited by the action of the phenol upon the mantle fibers.

At the end of 21 hours the root tips had a dull white luster, and were evidently dead. Upon examination all the cells were found to be plasmolyzed.

The radicles which had been for four hours in $n/188$ phenol were next examined and all stages of mitosis found. The cytoplasm of the cells of the outer layers exhibited a coarsely netted structure due to the formation of numerous small vacuoles. Occasional spireme nuclei were unduly enlarged, and in a very few cases the nuclei were constricted in one diameter, apparently by the development of a plasma mass as previously noted. The early stages of the spindle were very obscure, but during anaphase the fibers became quite distinct. A few cases of late anaphase occurred in which occasional chromosomes had not left the nuclear plate, but remained at one side of the spindle in line with the row of granules marking the early stages of cell plate formation (*fig. 7b*). A similar occurrence was observed by BLAZEK (**3**) in the cells of root tips of Pisum treated with benzol, and in *Vicia Faba* also by SCHRAMMEN (**27**), who says: "Eine nicht seltene Erscheinung bei der plötzlichen Einwirkung von hohen Temperaturen ist das Nichterfassen von Chromosomen durch die Spindelfasern und das Zurückbleiben einzelner Chromosomen bei dem raschen Transport zu den Spindelpolen." In a few cases the chromosomes at one pole of a spindle had diverged and formed two groups, to each of which a portion of the spindle extended (*fig. 7c*). SABLINE (**26**), who produced similar phenomena by the use of sulfate of quinine, says, "Sur quelques figures multipolaires on peut voir deux et quelquefois trois fuseaux." In the material treated with phenol, however, multipolar spindles occur so rarely that there seems to be no good ground for assuming that they are produced by the action of this reagent.

After an interval of 21 hours in $n/188$ phenol there were no mitoses. The nuclei were all in the resting stage and for the most part contained but a single nucleolus. General disorganization of the cells of the root tips had begun.

Tips of radicles were treated for 20 minutes with 10/94 normal phenol and then transferred to distilled water for four hours. At the end of this time all nuclear activity had ceased. In most cases the nucleus was enlarged, and in the field of the microscope appeared as a light area against the darker background of the cytoplasm of the cell (*fig. 7d*). The chromatin had almost entirely disappeared from the nuclear network. Very prominent in the nucleus appeared

one or two large and deeply staining nucleoli. After 24 hours under similar conditions there was little change in the appearance of the cells. Plasmolysis in occasional cells and the collapsed walls of the outer cell layer indicated that disorganization had begun.

Next 5/94 normal phenol was allowed to act for 20 minutes on root tips, which were then transferred to distilled water for four hours. There were no mitoses. In certain cells of the inner periblem and in some plerome cells elongating to form procambium, large vacuoles occurred, while many enlarged nuclei had partially collapsed. The general condition of the cells resembled very closely that obtaining after the exposure for four hours to the 10/94 normal solution. After 21 hours the root tips had made no further growth and were evidently dead. Disorganization of the cell structures, however, had not progressed so far as in the case of the material with 10/94 normal for the same time. Here again the nuclear area was enlarged, though very regular in form, and had a lighter hue than the surrounding cytoplasm. In the radicles treated for 20 minutes in the other dilutions employed, continuous growth occurred and development was apparently normal. After 20 minutes' treatment with $n/94$ and $n/188$ phenol, respectively, and then with distilled water for 45 hours, the root tips were fresh and crisp and had elongated at approximately the same rate as the distilled water controls. Mitoses were frequent, and the more active regions of the cytoplasm, particularly the achromatic figure, showed no injury as a result of the action of the phenol.

Phenol in common with most antiseptics is a marked protoplasmic poison. Certain results of its action were especially apparent in the cells of the root tips treated continuously with this reagent. The achromatic figure of the division nucleus in early anaphase was seriously injured and mitosis inhibited. The nuclear plate stage was apparently unusually sensitive to the action of the phenol, since the spindle fibers as a rule failed to function normally in drawing apart the daughter chromosomes. As previously noted, the development of small vacuoles in the cytoplasm appears to be a characteristic effect produced by the phenol. There was no amitotic division and no tendency toward the production of binucleate cells was observed.

In the material treated for 20 minutes with the stronger solutions and then with distilled water, the most striking modification was the

enlarged nuclear area surrounded by cytoplasm of a darker hue. Here also the cytoplasm usually contained small vacuoles, but neither amitosis nor abnormal division figures occurred. The general course of events under the action of the phenol seems to be the progressive decline of the cell functions, beginning in the most active and labile regions of the cytoplasm. The visible form changes are confined almost entirely to the enlargement of the nuclear area and the formation of numerous small vacuoles in the cytoplasm. The abnormalities due to the action of phenol are clearly differentiated from those occurring in the distilled water controls as previously described. The sphagnum controls were normal as usual.

THE EFFECT OF STRYCHNIN SULFATE

The preparation of the solution of strychnin sulfate and the manipulation of the material accorded closely with the plan pursued with the phenol. Five strengths of solution were used, which for convenience in comparison are expressed approximately in terms of percentage solutions, viz., 1, 0.5, 0.25, 0.1, and 0.01 per cent. In the first group of experiments the radicles were exposed to the constant action of the several solutions for intervals of 3, 6, and 24 hours. In the second group the root tips were treated with the various dilutions for ten minutes, and were then transferred to distilled water for the time intervals mentioned above. The usual controls were carried in sphagnum and in distilled water.

The 1 per cent solution was allowed to act on radicles for three hours. The tips were then a dull white color and had become flaccid. The cells were all plasmolyzed, and in the outer layers they were disintegrating.

From the external appearance after three hours' immersion in the 0.5 per cent solution death was inferred. However, in plerome and inner periblem a few nuclei were dividing. The outer cells were plasmolyzed and the cell walls were breaking down. In the inner cells the few scattered spireme nuclei and chromatic figures retained their normal shape and orientation in the presence of large vacuoles, one or more of which frequently occurred in the cytoplasm of these cells.

After three hours in the 0.25 per cent solution there were no mitoses.

The cytoplasm of many cells was plasmolyzed, while that of others exhibited a coarse web or net structure, but the cells themselves retained their shape and the outer layers were not disintegrated.

In the tips of radicles acted on by a 0.1 per cent solution for three hours all stages of division were observed. The achromatic figures were very distinct, and nuclei with two nucleoli occurred frequently. After six hours in this concentration the cells of the outer layers were dead and the cell walls had collapsed. There were some spireme nuclei in the inner periblem and a few nuclei were at anaphase. The large deeply staining nucleoli were prominent features of all nuclei not disorganized.

The cells of the outer layers of root tips in 0.01 per cent strychnin for three hours were mostly dead and collapsed. Division figures occurred occasionally in the plerome and were frequent in the inner periblem cells. The spindle fibers of anaphase were clear and distinct, but no polar caps were observed in spireme nuclei. The cell plate was regularly laid down in late anaphase and two nucleoli were frequent in resting nuclei. After five hours in the 0.01 per cent solution some division figures were present. The number of spireme nuclei in proportion to those in the later division stages was greater than at the end of three hours. Very few nuclei were in late anaphase. After 20 hours the area of dead cells included all but those of the inner periblem at some distance from the apex of the tip. No deformation of the nuclei occurred. Even in cells in which the cytoplasm was disorganized, the nuclei frequently retained their normal shape and general appearance. Growth and nuclear activity seemed to have been arrested by the gradual failure of cytoplasmic activity.

The radicles exposed for ten minutes to the strychnin solutions, then transferred to distilled water, were next considered. After ten minutes in 1 per cent solution and three hours in water the cells of the outer layer were dead. In many of the plerome and inner periblem cells containing division figures the cytoplasm was vacuolate, but the figures were not disturbed. All stages of division were observed, but there were no aberrant mitoses.

After six hours in this concentration further disintegration of the outer cells had taken place. Normal division figures persisted in the plerome and inner periblem cells, and a few rare spireme nuclei

showed clearly the polar caps. There were no abnormal structures. At the end of 24 hours there were no mitoses, all the cells were evidently dead.

In the tips exposed for ten minutes to the 0.5 per cent solution, and then placed in distilled water for three hours, mitotic figures occurred even in the outer cells of the periblem. The cytoplasm of these cells usually contained several large vacuoles and the nuclei were all normal in appearance. All stages of normal mitosis were abundant. After 6 hours little change in appearance was visible, but after 24 hours the cells of the outer layers were dead. However, all stages of normal division were abundant in the plerome and inner periblem.

Normal mitoses were abundant in the tips of radicles placed in the 0.25, 0.1, and 0.01 per cent strychnin solution for ten minutes, then in distilled water for 3, 6, and 24 hours. There was no evidence that the strychnin solution had exerted any harmful action in the last three concentrations, during ten minutes' exposure.

The data on the effects of strychnin on higher plants are not very extensive. According to PFEFFER (**25**), it has not been satisfactorily determined that alkaloids affect the protoplasm of plants. In his discussion of the effects of alkaloids in general, CZAPEK (**5**) says: "Für höhere Pflanzen stellte schon Knop an Mais fest, dass Chinin, Cinchonin, Morphin schädlich wirken, und auch hier gehören Chinin, Strychnin, Cocain zu den giftigsten Substanzen, während Morphin relativ schwach einwirkt (Marcacci)." Mosso (**14**) found that 0.05 per cent solutions of strychnin stimulated germination in *Phaseolus multiflorus*, but that more concentrated solutions retarded it. DAVENPORT (**6**) states that the protoplasm in the tentacles of Drosera is killed by the action of strychnin. He mentions also its retarding action on the germination of peas, corn, and lupines, but unfortunately the concentrations which exerted a harmful action were not given.

The action of alkaloids on Protozoa has been investigated by SCHÜRMAYER (**28**) and others, with results that apparently confirm the theory advanced by LOEW (**13**) that the action of alkaloids is mainly confined to the plasma of the ganglion cells. CLARK (**4**) found that species of fungi, notably Sterigmatocystis, as well as Aspergillus and Oedocephalum, grew and fruited in a saturated solution of strychnin sulfate. CLARK finds that his studies on the molds harmonize with the

theory of LOEW, and concludes that the fungi and bacteria are practically unharmed by this alkaloid, since they have no differentiation of nerve protoplasm. This line of reasoning carried logically forward would argue for the presence of protoplasmic structure in the higher plants which should be comparable with the nerve fibers of animals. Such structures have indeed been described by NEMEC (19) from root tips of Allium and *Vicia Faba*. In the latter plant the longitudinal protoplasmic strands of the large plerome cells of the root are regarded by NEMEC as bundles of fibrillae surrounded by a definite sheath and lying imbedded in a special plasma. NEMEC concludes that these fibrils are strands of protoplasm specialized for the conduction of traumatropic, geotropic, and other stimuli, and compares them, although with little apparent warrant, to the nerve fibers of animals. Since the protoplasm often develops a fibrillar structure in connection with other functions, it is not certain that the systems of fibrillae observed by NEMEC are specially adapted for the transmission of these stimuli, and therefore the portion of the protoplasm peculiarly sensitive to the action of alkaloids. ANDREWS (1) found that many marine plants, including Cladophora, Ectocarpus, and Polysiphonia, were uninjured by a solution of strychnin sulfate having one part in 1000 of water, but that a solution of the same having one part in 250 killed all the plants in 24 hours.

Although the experiments carried out with strychnin sulfate on *Vicia Faba* were far from satisfactory, they indicate clearly that this reagent is an active poison to the plant used. The cytoplasm first becomes vacuolate, and then degenerates in the outer cell layers, and this condition progresses toward the center of the root tip as the time of exposure to the strychnin solution is extended. It is planned to pursue this line of experimentation farther, in order to determine whether this reagent produces definite and characteristic form changes in the protoplasm.

Summary and conclusions

The cell studies here described were made in the hope of obtaining some further data on the physiology of toxic action. The work which has been done in this direction seems to be concerned more with the production and study of abnormal cell phenomena than with the com-

parison of cell activities under a series of abnormal conditions varying in intensity. It is well known that in a series of dilutions of a toxic substance growth diminishes usually as the concentration increases, and the end sought in these studies was to contrast cell activities under such abnormal conditions of development. No deliberate attempt was made to induce abnormal cell behavior.

The toxic solutions experimented with were (1) copper sulfate, a metallic base which readily ionizes; (2) phenol, a non-electrolyte; and (3) strychnin, an alkaloid presumably poisonous to protoplasm. First the rate of growth of radicles of *Vicia Faba* was determined in a series of concentrations of copper sulfate ranging from $n/20{,}000$ to $n/500{,}000$, then the number of hours required for growth to be reduced to the minimum was next observed in order to determine the range within which to choose material for study. Root tips grown in the above and intervening concentrations were examined at intervals ranging from 1 to 93 hours. Radicles were also subjected to the action of stronger solutions, $n/12$, $n/.320$, for intervals of 3 to 10 minutes, and the cells were examined after a lapse of 3 to 30 hours. The results indicate that the toxic effect was first felt in the kinoplasm of dividing cells, as shown by the loss of function and subsequent degeneration of the achromatic figure. Large vacuoles arose in the cytoplasm, frequently deforming achromatic figure and nucleus. Later the entire cytoplasm was disorganized. Development of the chromatic figure was consequently inhibited, but neither amitosis nor abnormal mitosis was observed. In the controls in distilled water, also, the cytoplasm became vacuolate; some of the nuclei were enlarged, and occasionally the formation of the cell plate was interrupted. In both copper sulfate solutions and distilled water the course of events was arrest of mitosis by loss of functions in the achromatic figure, followed by the death and disorganization of the cell contents.

The treatment of root tips with solutions of phenol ranging from $n/188$ to $10/94$ normal produced enlarged achromatic figures and caused the cytoplasm to become very coarsely netted or vacuolate. The chromatic figure was regularly formed and presented no special abnormalities. Neither amitosis nor binucleate cells occurred. The chromosomes were normal in number and structure. Spindle

formation was frequently inhibited, in consequence of which the chromosomes failed to separate normally. With the arrest of mitosis further development apparently ceased.

The experiments with strychnin were unsatisfactory. Solutions ranging from 0.01 to 1 per cent inhibited mitosis and disorganized the cytoplasm, causing the death of the cells. The nuclei were not deformed and the chromatic figures were normal. Strychnin seems to arrest cytoplasmic activity swiftly, without producing visible changes in the mitotic figure.

As a result of their investigations, certain authors state that nuclei can be made to divide amitotically through the influence of toxic solutions. Others, who used the same technic and methods, deny that such solutions produce amitosis, and find that in every case when division occurred the resulting nuclei were formed only by mitosis. BLAZEK (**3**) found that benzol caused the vacuoles in the cytoplasm to increase greatly in size; NEMEC (**18**) observed that chloroform and potassium nitrate produced granulation of the spindle fibers; WASIELEWSKI (**30**) ascribed doubling of the nucleoli to the action of chloral hydrate; WOYCICKI (**34**) states that ether prevented the formation of division walls in dividing cells; and WISSELINGH (**31**) found that under the influence of phenol the cell structures were poorly differentiated.

The authors just cited attributed the above-mentioned abnormalities solely to the action of the toxic substances used. In the experiments described in this paper all these abnormalities were observed in the toxicated material, and also in the controls grown in distilled water. These results appear to indicate that the action of distilled water is a factor which has been overlooked in interpreting the effect of toxic solutions on mitosis, and that numerous abnormalities ascribed to the action of toxic substances are not necessarily so produced.

CONCLUSIONS

1. The practice of growing controls in distilled water, common in certain physiological experiments, is open to serious objections, since these controls are themselves under abnormal conditions, and are subject to the same progressive decline of cell function as occurs in dilute toxic solutions, though at a slower rate.

2. Judged by its effect on mitosis, as compared with the effect of dilute solutions of copper sulfate, *distilled water is itself a toxic solution.* Apparently many abnormalities of cell behavior which have been attributed to the effect of toxic salts may be due instead to the osmotic action of the solution.

3. The achromatic structures organized from the kinoplasm are most sensitive to toxic action. Since the spindle fibers are reduced to a granular mass or otherwise disorganized, the further progress of division is inhibited.

4. Copper sulfate, phenol, and strychnin, under the conditions of these experiments, produce neither amitosis nor truly binucleate cells.

5. No structures occurred in the material studied which the most charitable interpretation could homologize with the large fusion nuclei containing double the normal number of chromosomes, produced, as stated by NEMEC, by a copper sulfate solution acting on radicles of *Vicia Faba.*

6. Doubling of the nucleolus is not a preparatory stage of amitosis, as stated by WASIELEWSKI.

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